Late depression of muscle excitability in humans after fatiguing stimulation

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1. Changes in muscle excitation and in isometric twitch force have been studied for up to 8 h after fatiguing stimulation of the human biceps brachii.

2. Within 10 s of a cessation of the 20 Hz fatiguing tetanus, the amplitudes of the M waves (muscle compound action potentials) had returned to control values, whereas the twitch forces were reduced in all subjects. The M waves then decreased in amplitude over the next 3 h, reaching a mean value that was 42·4 ± 18·6% of control levels (means ± S.E.M.; $P < 0{\cdot}001$).

3. By 8 h, the mean M wave amplitude had recovered to 93·8 ± 33·3% of control levels, while the corresponding mean twitch force was 104·1 ± 36·9%.

4. The cellular mechanism responsible for the depression of the M wave is presently unknown, but it is likely to be postsynaptic and may involve Na^+ channels.

The muscle compound action potential (M wave) has been used extensively as one of the indices of fatigue and recovery in both human and animal studies. Thus, the M wave, being the sum of the single muscle fibre action potentials elicited by electrical stimulation, provides important information regarding the net effects of ion fluxes, Na^+–K^+ pump activity and neuromuscular transmission. However, although there have been many studies of muscle fatigue, few have been concerned with long-term changes in muscle excitation. In most experiments the observations have been terminated within a few minutes of the end of the fatiguing contractions, at a time when the M wave has returned to control values.

The impetus for the present study came from the investigation of the M wave potentiation which accompanies repetitive muscle excitation (Fitch & McComas, 1985) and has been attributed to enhanced Na^+–K^+ pump activity (Hicks & McComas, 1989). In such experiments we were surprised to observe that, after initial recovery of the M wave, the response underwent a gradual decline; however, neither the full extent nor the duration of the decline were measured (Cupido, Galea & McComas, 1996). Of other investigators, only Länngergren, Larsson & Westerblad (1989) appear to have noticed such late depression of the M wave, and this was in single motor units of the rat tibialis anterior muscle. In contrast to the situation for muscle excitability, there have been several investigations of the recovery of force after fatiguing stimulation and there is general agreement that the responses to single shocks and low-frequency stimulation may be depressed for several hours; this phenomenon has been termed 'low-frequency fatigue' and is attributed to impairment of excitation–contraction coupling (Edwards, Hill, Jones & Merton, 1977). It is the documentation of the long-term changes in muscle membrane excitation, as expressed by the M wave, which has been the objective of the present study.

A preliminary account of these results has appeared elsewhere (Galea, McFadden, Cupido & McComas, 1993).

METHODS

Subjects

Nine male volunteers, aged 18–32 years (mean age ± S.D., 22·8 ± 4·0 years) participated in the study and gave their written informed consent. Although apparently healthy, all nine subjects led sedentary lives and did not participate in any sports or other exercise. The study carried the approval of the McMaster University Ethics Committee.

Stimulating and recording arrangements

The subjects lay supine on a bed with the right elbow flexed at 90 deg and with the wrist attached to a U-shaped force transducer; the latter was constructed of aluminium and the segment to which the forearm was attached measured 7·5 cm × 4·0 cm. The system was mounted on a braced metal upright, and fixed to the bed. The transducer had a resonant frequency of 5·5 kHz and was linear over the range of forces encountered in the study.

M wave responses were recorded over the main innervation zone of the biceps brachii and a common reference electrode was placed on the forearm just below the elbow. Both recording electrodes, together with the ground electrode on the back of the hand, were adhesive Ag–AgCl ECG electrodes (34 mm × 22 mm; Sentry

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Medical Products, Irvine, CA, USA). The M wave responses were fed through amplifiers with pass-bands of 5–10 kHz and, together with the amplified force signals, were displayed on a variable persistence oscilloscope (Model 141B, Hewlett Packard Ltd). The electrical and mechanical responses were also entered into a digital acquisition and analysis system (CODAS, Dataq Instruments Inc., Akron, OH, USA).

The stimuli were rectangular voltage pulses, 0·1 ms in duration, and were applied to intramuscular nerve branches in the rostral pole of the biceps muscle through two lead plate surface electrodes (35 mm × 25 mm). The stimulating electrodes were fastened to the skin with adhesive tape and held in place for the duration of the experiment with a lightly bound gauze bandage; care was taken not to compress the underlying nerve twigs. The stimulator (Model 3072, Digitimer Ltd, Welwyn Garden City, Herts, UK) was controlled by a digital timing device (Model 3920 digitimer, Digitimer Ltd) via a gated pulse generator (Model 2521, Digitimer Ltd).

Experimental procedure

With the subject relaxed, four supramaximal stimuli, 0·1 ms in duration and separated by 10 s, were used to obtain control values of M wave size and twitch force for comparison with the responses to single shocks during the recovery period. The stimulus was periodically checked, using the M wave as an index, to ensure that it remained supramaximal throughout the experiment (intensities, 14–55 mA).

After the control observations had been made, a 20 Hz tetanus was started and maintained until the tetanic force had dropped to 50% or less of the initial value (mean tetanus duration, 4·7 ± 1·7 min).

The tetanus was then stopped and the recovery process was examined with single testing stimuli; the interval between successive stimuli was initially 10 s, and was gradually increased to 15 min. During this 2 h period, the subjects lay quietly on the bed. Additional responses were elicited at 60 min intervals for a further 6 h; during this period the arm was immobilized in a sling, and the subject was allowed to leave the laboratory.

To exclude the possibility of diurnal or other variations in muscle responsiveness, five of the subjects participated in additional experiments, each of 8 h duration. Throughout this period M waves were monitored by single supramaximal stimuli. These experiments differed from the previous ones in that there was no tetanic stimulation.

Measurements

Using the CODAS system (see above) measurements were made of the peak-to-peak amplitudes of the M waves recorded in the innervation zone of the biceps muscle. In addition, the areas of the M waves and the time intervals between the negative and positive peaks were also determined; however, because the shock artifact tended to be large, the measurements of area were sometimes unreliable and only those of amplitude have been presented. Twitch force was measured in the usual way (Vandervoort, Quinlan & McComas, 1983).

Statistical analysis

Differences between selected mean values were analysed with the Student's t test, while correlation coefficients were calculated using the Pearson product correlation coefficient. Mean values in the text are given with their standard deviations, while standard errors of the means are shown in Fig. 1.

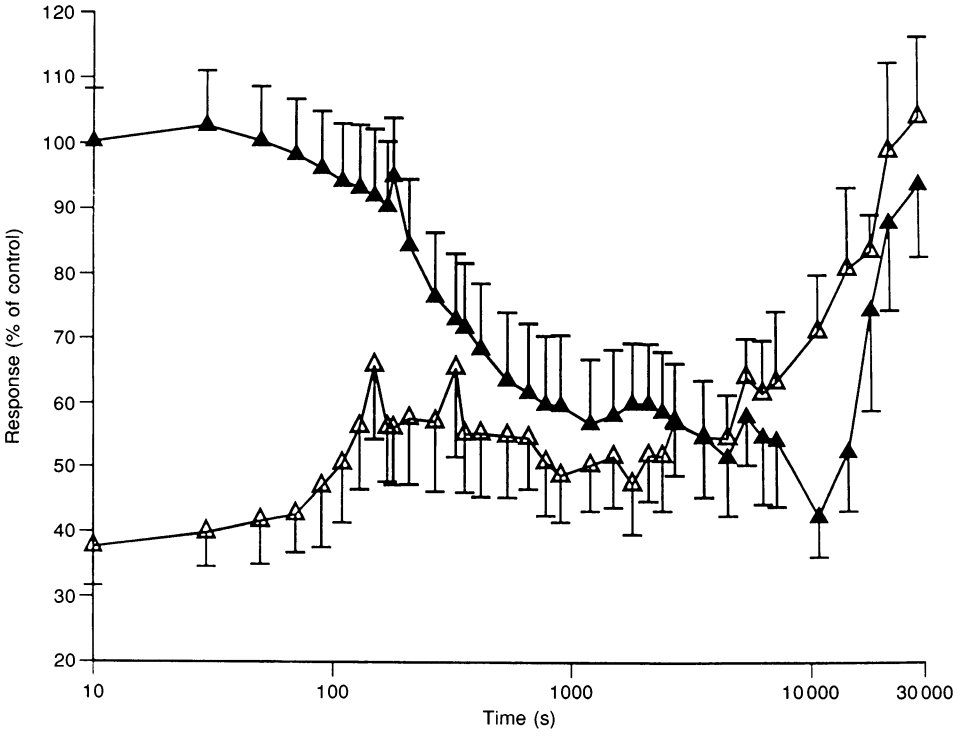

Figure 1. Depression and recovery of M wave amplitude and force

Mean values ± S.E.M. are shown for the M wave (▲) and for twitch force (△) in the 9 subjects following fatiguing stimulation at 20 Hz.

RESULTS

Fatigue development

Following initial potentiation, which lasted for approximately 30 s, tetanic force quickly decreased, with 50% of mean force being lost by 130 s.

In keeping with previous observations (Cupido *et al.* 1996), the M waves also potentiated early in the tetanus, with increases occurring in both amplitude and area. The mean amplitude then fell and there was an increase in the duration of the response.

M wave changes after tetanization

After the 20 Hz tetanus ceased, the M waves quickly resumed their control values; by 3 min, however, the responses began to diminish (Fig. 1). The decline in mean value continued for almost 3 h (10 000 s), the mean amplitude reaching a value which was 42·4 ± 18·6% of control levels ($P < 0{\cdot}001$). The M waves then increased and, by approximately 5·5 h (20 000 s), had almost attained control values. Figure 2 shows the most striking example of the changes in the M wave, recorded in one of the subjects.

In two subjects an additional brief tetanus was delivered at the time of maximum M wave depression in order to see whether the latter was reversible. However, the imposed tetanus caused little enhancement of the M wave (approximately 10%) and appeared to prolong the depression.

Additional experiments were performed to rule out the possibility that the source of the changes in M wave amplitude was caused by diurnal or other fluctuations in muscle fibre excitability. In these experiments, performed on five subjects, no tetanus was delivered but the usual observations of M wave size were made over a period of several hours. No significant change in M wave amplitude occurred throughout this time.

Changes in twitch force during recovery

The recovery of force appeared to take place in three stages. Thus, in Fig. 1 it can be seen that there was an initial phase, in which approximately 60% of force had returned by 150–300 s. There was then a slight decline, followed by the third phase, one of slow recovery.

DISCUSSION

Observations of muscle excitation in fatigue experiments are usually terminated within a few minutes of the end of the contractions, at a time when the M wave has returned to its control size (e.g. Hultman & Sjoholm, 1983). The implication in such studies is that excitability of the surface membrane is unlikely to change, even though a depression of force to single shocks or low-frequency stimulation is known to persist for up to 24 h (Edwards *et al.* 1977). In the present investigation, however, it has been shown that the M wave undergoes a marked reduction in amplitude which may

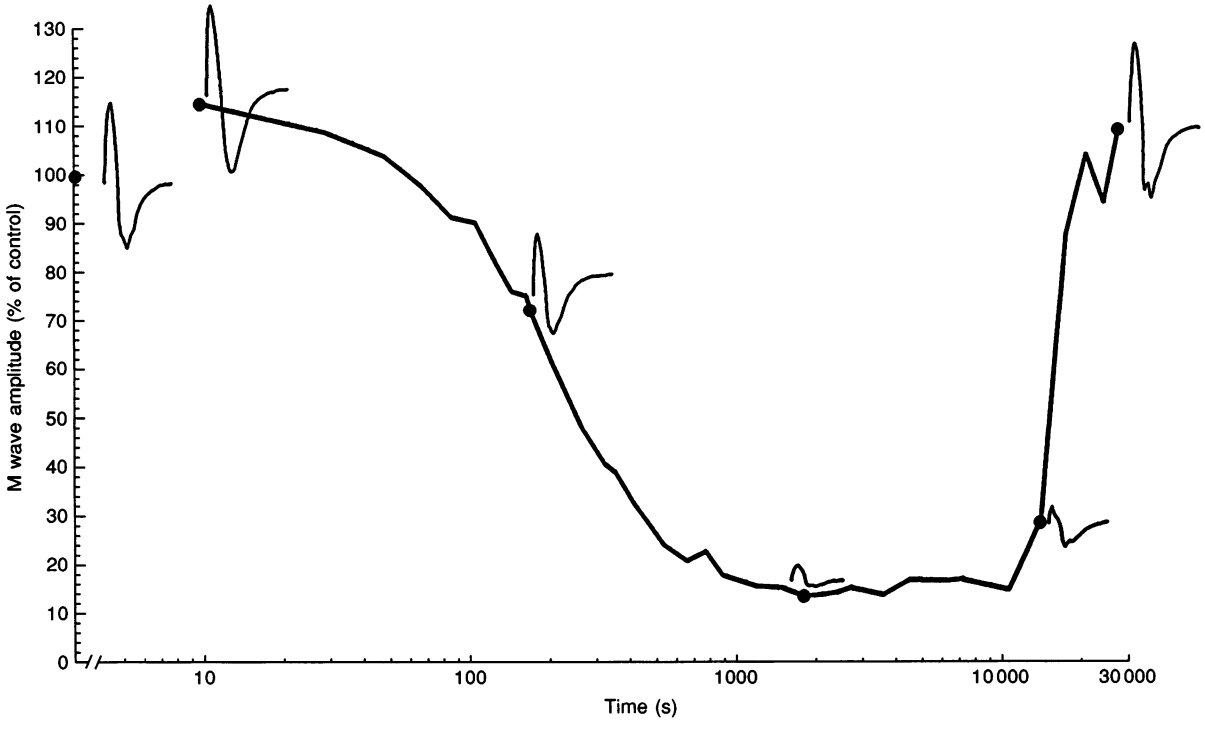

Figure 2. Severe M wave depression in a subject

The marked depression and subsequent recovery of M wave amplitudes are shown in a single subject following 20 Hz fatiguing stimulation, together with recordings of M waves at critical time points. Each recording was started 4 ms after stimulus delivery.

persist for several hours. In animal studies, the only previous mention of diminished muscle excitability after fatigue appears to be that of Lännergren *et al.* (1989), who found evidence of this phenomenon in single motor units of the rat tibialis anterior.

Possible causes of M wave depression

The cellular mechanism responsible for the depression of the M wave could be impaired neuromuscular transmission or decreased excitability of the muscle fibre plasmalemma. Direct stimulation of muscle fibres would provide one means of deciding between these possibilities, but is not easily achieved in human subjects. To ensure that intramuscular nerve branches were not inadvertently stimulated, because of their having lower thresholds than the muscle fibres, it would be necessary to block neuromuscular transmission completely. Also to achieve supramaximal stimulation of an entire muscle would require very large currents (Merton, Hill & Morgan, 1981), which would not only be painful but could damage the skin.

Although the possibility of impaired neuromuscular transmission cannot be tested directly, it seems, on theoretical grounds, to be an unlikely cause of the changes in the M wave. In the first place, the safety factor of the human neuromuscular junction to single shocks or low-frequency stimulation is appreciable, largely due to a high concentration of postsynaptic Na^+ channels (Flucher & Daniels, 1989), especially in type II fibres (Ruff & Whittlesey, 1992). Second, in experiments from our laboratory, we have employed the same fatiguing protocol and animal preparation as Lännergren and colleagues (1989; see above) and have observed a fall in mean resting membrane potential in rat tibialis anterior muscle fibres which is usually commensurate with the depression of the M waves (S. Kuiack & A. J. McComas, unpublished observations). Such a finding would clearly place the site of dysfunction beyond the neuromuscular junction.

In relation to the muscle fibre plasmalemma, there are at least three possible mechanisms which might account for the changes observed in the M wave. These mechanisms are: reduced Na^+–K^+ pump activity, impaired inactivation of Na^+ channels and physical damage to the muscle membrane.

In relation to the first possibility, it is well established that Na^+–K^+ pump activity is ultimately responsible for maintaining the entire resting potential of the muscle fibre, by compensating for the leakiness of the membrane to Na^+. In addition, the pump provides a small part (−5 to −10 mV) of the resting potential directly, through its electrogenic action (Hicks & McComas, 1989). Clearly then, if the pump were to fail, depolarization of the muscle fibres and depression of the M wave would result. Arguing against this possibility, however, is the fact that the energy stores of the muscle, necessary for driving the pump, return to normal within a few minutes after fatiguing contractions, and there is a similarly rapid clearance of fatigue-induced metabolites from the muscle (cf. Boska, Moussavi, Carson, Weiner & Miller, 1990). Also, the Na^+–K^+ pump has a large reserve and only 2–6% of its capacity is utilized in resting muscle (Clausen, 1986).

Although altered Na^+ channel inactivation is, at first sight, an equally unlikely explanation for M wave depression, there are two genetic disorders in which the Na^+ channel is abnormal and marked reductions in M wave amplitude take place over a period of many minutes, following voluntary or stimulated contractions (McManis, Lambert & Daube, 1986). In both these conditions, hyperkalaemic familial periodic paralysis and paramyotonia congenita, the genetic mutation prevents the normal fast inactivation of the Na^+ channels from taking place (Lehmann-Horn, Küther, Ricker, Grafe, Ballanyi & Rüdel, 1987; Fontaine *et al.* 1990). It is not clear, however, what the role of slow inactivation in these disorders is (Cummins & Sigworth, 1996), and it is possible that it is this type of inactivation which is involved in the M wave depression found in the present study.

The final possibility, that of plasmalemmal damage, should also be considered in view of the degenerative changes which can take place in muscle fibres after intensive exercise. One such change is 'streaming' of the Z-disc (Armstrong, Warren & Warren, 1991). The attachment of the Z-disc to the plasmalemma might induce minute tears in the latter.

Changes in twitch force during recovery

The complex three-phase recovery of twitch force has been attributed elsewhere to differences in time course between the force-potentiating and force-fatiguing processes (Garner, Hicks & McComas, 1989). Again, the prolonged reduction in twitch force observed in the present investigation is consistent with other studies in which the responses to single shocks or low-frequency stimuli have been depressed for up to 24 h (Edwards *et al.* 1977). Since high-frequency stimuli can elicit normal force, the low-frequency fatigue has been attributed to defective excitation–contraction coupling. In keeping with this explanation, a reduction in the release of Ca^{2+} from the sarcoplasmic reticulum has been demonstrated in single mammalian muscle fibres during the recovery period following tetanization (Westerblad, Duty & Allen, 1993).

A surprising feature of the present study was the discrepancy between the M wave and force during the later part of the recovery period. For example, at 10 000 s, the mean force was 70% of normal while the mean M wave amplitude was only 42%. However, Sandow (1952) was the first to show, by altering the ionic composition of the bathing fluid, that the externally recorded compound action potential of frog sartorius muscle could be reduced by 50% or more without any decline in twitch force. Although a similarly large safety factor for excitation–contraction coupling may have been operating during the recovery period in the present experiments, we cannot exclude the possibility that some of the loss of force in the inactive subjects resulted from the smallness of their M waves.

In conclusion, we have provided evidence of a novel phenomenon by which the muscle fibre excitation, as measured by the M wave amplitude, remains depressed for up to 6 h following low-frequency stimulation. Further studies would be useful in showing whether or not similar depression occurs after voluntary contractions, and also the effect, if any, of exercise training on the phenomenon. Although the present study does not identify the mechanism, the finding of a long-term depression in muscle fibre excitability after fatiguing stimulation is one which is likely to lead to new insights into the biochemistry of the plasmalemma.

ARMSTRONG, R. B., WARREN, G. L. & WARREN, J. A. (1991). Mechanisms of exercise-induced muscle fibre injury. *Sports Medicine* **12**, 184–207.

BOSKA, M. D., MOUSSAVI, R. S., CARSON, P. J., WEINER, M. W. & MILLER, R. G. (1990). The metabolic basis of recovery after fatiguing exercise of human muscle. *Neurology* **40**, 240–244.

CLAUSEN, T. (1986). Regulation of active Na^+–K^+ transport in skeletal muscle. *Physiological Reviews* **66**, 542–580.

CUMMINS, T. R. & SIGWORTH, F. J. (1996). Comparison of slow inactivation in HYPP-T698M and F1304Q mutant rat skeletal muscle Na^+ channels. *Biophysical Journal* **70**, A132.

CUPIDO, C. M., GALEA, V. & MCCOMAS, A. J. (1996). Potentiation and depression of the M wave in human biceps brachii. *Journal of Physiology* **491**, 541–550.

EDWARDS, R. H., HILL, D. K., JONES, D. A. & MERTON, P. A. (1977). Fatigue of long duration in human skeletal muscle. *Journal of Physiology* **272**, 769–778.

FITCH, S. & MCCOMAS, A. (1985). Influence of human muscle length on fatigue. *Journal of Physiology* **362**, 205–213.

FLUCHER, B. E. & DANIELS, M. P. (1989). Distribution of Na^+ channels and ankyrin in neuromuscular junctions is complementary to that of acetylcholine receptors and the 43 kd protein. *Neuron* **3**, 163–175.

FONTAINE, B., KHURANA, T. S., HOFFMAN, E. P., BRUNS, G. A. P., HAINS, J. L., TROFATTER, J. A., HANSON, M. P., RICH, J., MCFARLANE, H., MCKENNA YASEK, D., ROMANO, D., GUSELLA, J. F. & BROWN, R. H. (1990). Hyperkalemic periodic paralysis and the adult muscle sodium channel α-subunit gene. *Science* **250**, 1000–1002.

GALEA, V., MCFADDEN, L., CUPIDO, C. & MCCOMAS, A. J. (1993). Delayed depression of human muscle excitability following fatigue. *Canadian Journal of the Neurological Sciences* **20**, 359.

GARNER, S. H., HICKS, A. L. & MCCOMAS, A. J. (1989). Prolongation of twitch potentiating mechanism throughout muscle fatigue and recovery. *Experimental Neurology* **103**, 277–281.

HICKS, A. & MCCOMAS, A. J. (1989). Increased sodium pump activity following repetitive stimulation of rat soleus muscles. *Journal of Physiology* **414**, 337–349.

HULTMAN, E. & SJOHOLM, H., (1983). Electrogram, force and relaxation time during and after continuous electrical stimulation of human skeletal muscle *in situ*. *Journal of Physiology* **339**, 33–40.

LÄNNERGREN, J., LARSSON, L. & WESTERBLAD, H. (1989). A novel type of delayed tension reduction observed in rat motor units after intense activity. *Journal of Physiology* **412**, 267–276.

LEHMANN-HORN, F., KÜTHER, G., RICKER, K., GRAFE, P., BALLANYI, K. & RÜDEL, R. (1987). Adynamia episoda hereditaria with myotonia: a non-inactivating sodium current and the effect of extracellular pH. *Muscle and Nerve* **10**, 363–374.

MCMANIS, P. G., LAMBERT, E. H. & DAUBE, J. R. (1986). The exercise test in periodic paralysis. *Muscle and Nerve* **9**, 704–710.

MERTON, P. A., HILL, D. K. & MORTON, H. B. (1981). Indirect and direct stimulation of fatigued human muscle. In *Human Muscle Fatigue: Physiological Mechanisms*, ed. PORTER, R. & WHELAN, J., *CIBA Foundation Symposium*, vol. 82, pp. 120–129. Wiley, Chichester.

RUFF, R. L. & WHITTLESEY, D. (1992). Na^+ current densities and voltage dependence in human intercostal muscle fibres. *Journal of Physiology* **458**, 85–97.

SANDOW, A. (1952). Excitation–contraction coupling in muscular response. *Yale Journal of Biology and Medicine* **25**, 176–201.

VANDERVOORT, A. A., QUINLAN, J. & MCCOMAS, A. J. (1983). Twitch potentiation after voluntary contraction. *Experimental Neurology* **81**, 141–152.

WESTERBLAD, H., DUTY, S. & ALLEN, D. G. (1993). Intracellular calcium concentration during low-frequency fatigue in isolated single fibers of mouse skeletal muscle. *Journal of Applied Physiology* **75**, 382–388.

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